*Korean J Physiol Pharmacol*
*Vol 16: 293−296, October, 2012*
*http://dx.doi.org/10.4196/kjpp.2012.16.5.293*

# Effect of Intensity of Unconditional Stimulus on Reconsolidation of Contextual Fear Memory

**Chuljung Kwak[1], Jun-Hyeok Choi[1], Joseph T. Bakes[3], Kyungmin Lee[2], and Bong-Kiun Kaang[1,3]**

*[1]National Creative Research Initiative Center for Memory, Department of Biological Sciences, College of Natural Sciences, Seoul National University, Seoul 151-747, [2]Department of Anatomy, Graduate School of Medicine, Brain Science & Engineering Institute, Kyungpook National University, Daegu 700-842, [3]Department of Brain and Cognitive Sciences, College of Natural Sciences, Seoul National University, Seoul 151-747, Korea*

**Memory reconsolidation is ubiquitous across species and various memory tasks. It is a dynamic process in which memory is modified and/or updated. In experimental conditions, memory reconsolidation is usually characterized by the fact that the consolidated memory is disrupted by a combination of memory reactivation and inhibition of protein synthesis. However, under some experimental conditions, the reactivated memory is not disrupted by inhibition of protein synthesis. This so called "boundary condition" of reconsolidation may be related to memory strength. In Pavlovian fear conditioning, the intensity of unconditional stimulus (US) determines the strength of the fear memory. In this study, we examined the effect of the intensity of US on the reconsolidation of contextual fear memory. Strong contextual fear memory, which is conditioned with strong US, is not disrupted by inhibition of protein synthesis after its reactivation; however, a weak fear memory is often disrupted. This suggests that a US of strong intensity can inhibit reconsolidation of contextual fear memory.**

**Key Words: Boundary condition, Contextual fear memory, Memory strength, Protein synthesis inhibitor, Reconsolidation**

## INTRODUCTION

Memory reconsolidation refers to the re-stabilization of long-term memory [1,2] after its reactivation. Various signaling pathways involved in *de novo* protein synthesis are required for this re-stabilization [3]. A number of researchers have used the fear-memory paradigm to characterize memory reconsolidation. Memory reconsolidation is generally perceived to be processed when an amnestic agent paired with memory reactivation disrupts the consolidated memory. In most cases, fear memories are reactivated by presentation of a conditional stimulus (CS). Anisomycin-a protein synthesis inhibitor-is most commonly used as the amnestic agent to disrupt reactivated fear memory.

However, in experimental conditions, the use of a protein synthesis inhibitor does not always impair the reactivated fear memory [4-7]. Several experimental variables influence the reconsolidation of memory after reactivation; some of these include memory strength [4,5], age of memory [8] and specificity of the CS [9]. A strong fear memory can block the reconsolidation of fear memory. This is consistent with the hypothesis that memory reconsolidation is the process by which memories are modified or updated. In auditory and contextual fear memories, increasing the number of pairings of conditional stimuli with unconditional stimuli blocks reconsolidation of fear memory [4,5]. In previous studies, researchers increased the number of unconditional stimulus (US) to give subjects a strong fear memory.

Along with the number, intensity of US also critical factor for memory strength. In concextual fear memory with mouse, several shock intensitiy have been commonly used (mostly, 0.4∼0.8 mA). Because those various US intensities did not substantially affect freezing level of mouse, less attention have been paid to an effect of US intensity on memory strength and reconsolidation. Therefore, it is required investigate whether US intensity also affects reconsolidation of contextual fear memory. In this study, we investigated the effect of the intensity of US on the reconsolidation of contextual fear memory. We observed that strong US intensity inhibits the reconsolidation of contextual fear memory, suggesting that in an experimental setting, the intensity of US is an important factor for contextual fear memory reconsolidation.

Received March 30, 2012, Revised June 11, 2012,
Accepted September 20, 2012

Corresponding to: Bong-Kiun Kaang, Department of Biological Sciences, College of Natural Sciences, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-747, Korea. (Tel) 82-2-880-9024, (Fax) 82-2-884-9577, (E-mail) kaang@snu.ac.kr



**ABBREVIATIONS:** US, unconditional stimulus; CS, conditional stimulus; NMDA, N-methyl-D-aspartate.

## METHODS

### *Animals*

All experiments were conducted on adult male mice as done in our previous study [10]. The mice were 9∼11 weeks old. They were housed under a 12:12 h light-dark cycle, with food and water provided *ad libitum*. The investigator was blinded to the experimental group while conducting behavioral experiments.

### *Contextual fear memory consolidation*

For contextual fear conditioning, the mice were placed in a fear conditioning chamber (Coulbourn Instruments) for 3 min. After 2 min, a single electrical foot shock was given. The mice were placed in the conditioning chamber for an additional 1 min after the electrical foot shock. To test the effect of anisomycin on memory consolidation (Fig. 1), a 0.4 mA electrical foot shock was given. We assessed the fear memory for 5 min in the same chamber the next day.

### *Contextual fear memory reconsolidation*

The experimental procedure for fear conditioning for reconsolidation was the same as that for consolidation, with the exception of the strength of the electrical foot shock. To test the differential effect of memory strength on contextual fear memory reconsolidation, 0.4-mA and 0.8-mA electrical foot shocks were used as weak and strong US, respectively. One day after the electrical conditioning, the mice were placed in the same fear chamber for 3 min. Then, anisomycin was immediately administrated in an intraperitoneal manner. The fear memory was tested the following day in the same chamber for 5 min.

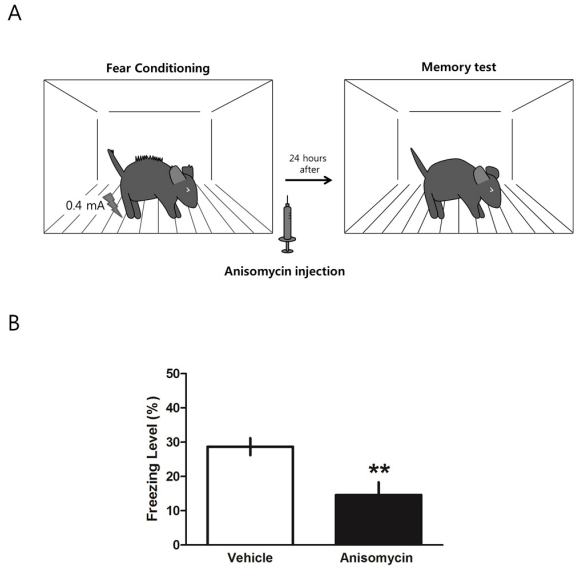


**Fig. 1.** Administration of protein synthesis inhibitor blocks the consolidation of contextual fear memory. (A) Experimental design. (B) Effect of protein synthesis inhibito on consolidation of contextual fear memory. Bars represent the means±SEM of freezing levels assessed 24 h after conditioning. Compared to the vehicle group, the anisomycin group showed impaired freezing level (vehicle, n=9; anisomycin, n=8, unpaired t test, **p=0.0060).

### *Drug*

Anisomycin (Sigma) was first dissolved in HCl. Later, NaOH and PBS were added to the solution to adjust the pH between 7.0 and 7.4. The concentration of the final anisomycin dose was 150 mg/kg.

## RESULTS

Protein synthesis is the principal process involved in stabilization of memory consolidation and reconsolidation. Hence, to inhibit protein synthesis, we systemically injected adult male mice with anisomycin (dose, 150 mg/kg), a protein synthesis inhibitor. We chose the dose and the route of administration for their known effectiveness. Systemic injection of anisomycin 150 mg/kg inhibited >90% of the protein synthesis in the brain during the first 2 h [10]. To investigate the disruptive effect of anisomycin injection on the memory formation process, we first examined whether systemic injection impaired memory consolidation (Fig. 1). Anisomycin was immediately administered after contextual fear-memory conditioning (Fig. 1A). Consistent with the results of previous studies, our results showed that anisomycin administration inhibited consolidation of contextual fear memory (Fig. 1B). Therefore, in our experimental setting, administration of anisomycin disrupted the consolidation of long-term memory.

Next, we investigated the effects of training intensity on the reconsolidation of contextual fear memory by administering the mice with 0.4 mA (weak US) and 0.8 mA (strong US) foot shocks. The mice were conditioned with a single foot shock and were re-exposed to the stimulus for 3 min after 24 h. Immediately after the re-exposure, we administered anisomycin to block fear memory reconsolidation. We assessed the contextual fear memory for 5 min the next day (Fig. 2A). Interestingly, anisomycin administration blocked reconsolidation only when the animals were trained with the weak shock (0.4 mA) (Fig. 2B left). The mice that were conditioned with the strong shock (0.8 mA) did not show any impairment of fear memory (Fig. 2B right). These results suggest that the intensity of the US determines whether reactivation of the fear memory is influenced by the protein synthesis inhibitor.

## DISCUSSION

As mentioned in previous studies, similar to the effects of boundary condition on reconsolidation, strong fear memories also block the reconsolidation of auditory fear memory [5] and contextual fear memory paradigm [4]. These results suggest that strong fear conditioning blocks the reconsolidation of fear memory. This finding is consistent with the view that memory reconsolidation is a dynamic process in which memory is either modified or updated [1,12]. These findings show that the resistance of strong fear memory to modification can be attributed to the absence of a reconsolidation process. However in some cases, when such a strong conditioning protocol is not used, the fear memory was not reconsolidated [7]. Therefore, this discrepancy is not conclusively solved.

In fear-memory conditioning, the intensity of the elec-

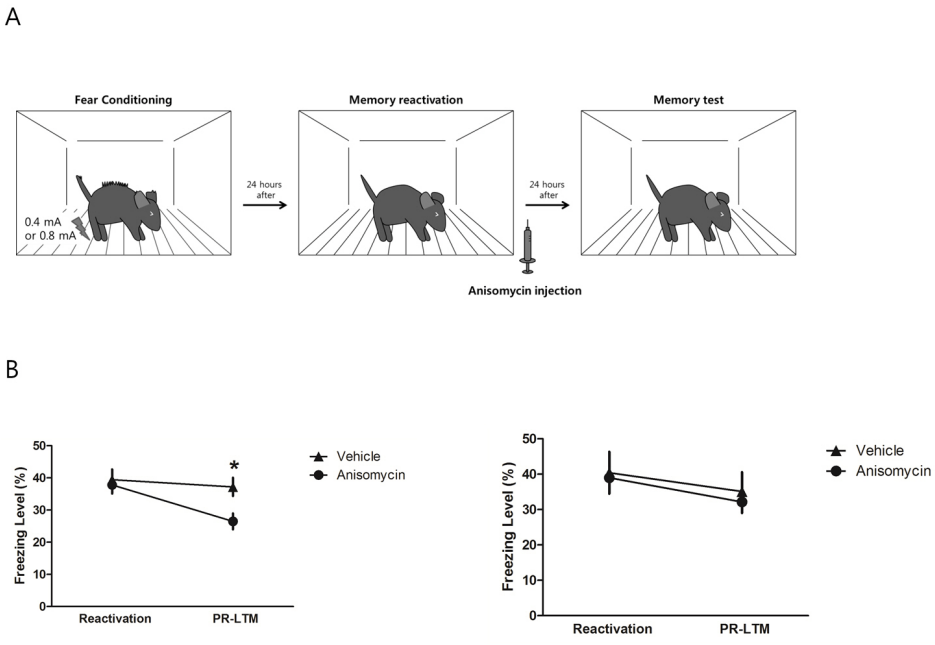


**Fig. 2.** Protein synthesis inhibitor has differential effects on reconsolidation of contextual fear memory according to memory strength. (A) Experimental design. (B) Effect of protein synthesis inhibitor on reconsolidation of weak contextual fear memory (lest panel) and strong fear memory (right panel). Bars represent the means±SEM of freezing levels assessed at reactivation and 24 h after reactivation. Protein synthesis inhibitor blocks reconsolidation of weak fear memory, but not strong fear memory (left panel, vehicle, n=12; anisomycin, n=12; 2-way ANOVA test, $*p < 0.05$) and strong contextual fear memory (vehicle, n= 6; anisomycin, n= 6; 2-way ANOVA test, $p > 0.05$).

trical foot shock is also an important factor in determining the strength of fear memory. In this study, we explored the effect of shock intensity on reconsolidation of fear memory. Previous studies increased the number of pairings of conditional stimuli with unconditional stimuli to give the animal a stronger fear memory. Instead of increasing the number of pairings, we increased the intensity of electrical foot shock. We observed that the reactivated fear memory was disrupted by administration of anisomycin, but only when the mice were conditioned with weak US. This implies that US intensity is also an important factor for reconsolidation of contextual fear memory.

In contextual fear memory protocol, conditioning with 0.8 mA of electrical shock has been commonly used. The novel point of our research is that even for animals conditioned with a widely used unconditional stimulus, this might be too strong to undergo reconsolidation. Therefore, our results provide researchers with the experimental conditions in which contextual fear memory will undergo reconsolidation.

Further research is required to investigate the brain region involved with boundary conditions of contextual fear memory. In previous studies, the down-regulation of N-methyl-D-aspartate (NMDA) receptor is suggested to be the mechanism underlying the boundary condition [5]; this is consistent with the finding that the NMDA receptor initiates reconsolidation of auditory fear memory [13]. Consolidation and reconsolidation of auditory fear are largely dependent on the amygdala, and the NMDA receptor is down-regulated within the hippocampus. This means that brain regions involved in the boundary condition are dissociated with the brain regions engaged in memory reconsolidation. The brain region that consolidates and reconsolidates contextual fear memory is the hippocampus [14,15]. Therefore, it is important to investigate whether the hippocampus is involved with both reconsolidation and boundary condition or another brain region is involved with the boundary condition of contextual fear memory.

Modification of our experimental conditions could possibly induce reconsolidation of a strong fear memory. The appropriate memory reactivation to induce a labile state of consolidated fear memory differs according to the training protocol [4]. In a previous study, prolonged duration of reactivation allowed the strong fear memory to be sensitive to a protein synthesis inhibitor. Instead of shock intensity, increased the number of electrical shocks to give the animal a stronger training [4]. Consistent with our findings, they also observed that stronger training blocks reconsolidation of contextual fear memory. It is interesting to note that a prolonged duration of reactivation made the reactivated strong fear memory sensitive to anisomycin. In this regard, the re-exposure provided in our study may not have been strong enough to successfully reactivate the strong fear memory. There is a possibility that a strong memory conditioned by a stronger US may also require a longer retrieval duration, as in the case of a strong memory conditioned by repeated US. Therefore, whether a stronger shock induced-boundary condition can be abolished by modifying of the re-exposure protocol, e.g., by prolonging the duration, needs to be investigated.

## ACKNOWLEDGEMENTS

This work was supported by the National Creative Research Initiative Program of the Korean Ministry of Science and Technology. CK and JHC are supported by BK21 fellowships. CK is also supported by the Lotte fellowship. K.L. was supported by Basic Science Research Program (2011-0028240) through National Research Foundation of Korea. B.K.K. is supported by the National Honor Scientist Program.

## REFERENCES

1. **Nader K, Hardt O.** A single standard for memory: the case for

reconsolidation. *Nat Rev Neurosci.* 2009;10:224-234.
2. **Nader K, Schafe GE, Le Doux JE.** Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature.* 2000;406:722-726.
3. **Tronson NC, Taylor JR.** Molecular mechanisms of memory reconsolidation. *Nat Rev Neurosci.* 2007;8:262-275.
4. **Suzuki A, Josselyn SA, Frankland PW, Masushige S, Silva AJ, Kida S.** Memory reconsolidation and extinction have distinct temporal and biochemical signatures. *J Neurosci.* 2004;24: 4787-4795.
5. **Wang SH, de Oliveira Alvares L, Nader K.** Cellular and systems mechanisms of memory strength as a constraint on auditory fear reconsolidation. *Nat Neurosci.* 2009;12:905-912.
6. **Cammarota M, Bevilaqua LR, Medina JH, Izquierdo I.** Retrieval does not induce reconsolidation of inhibitory avoidance memory. *Learn Mem.* 2004;11:572-578.
7. **Biedenkapp JC, Rudy JW.** Context memories and reactivation: constraints on the reconsolidation hypothesis. *Behav Neurosci.* 2004;118:956-964.
8. **Lattal KM, Abel T.** Behavioral impairments caused by injections of the protein synthesis inhibitor anisomycin after contextual retrieval reverse with time. *Proc Natl Acad Sci USA.* 2004;101:4667-4672.
9. **Doyère V, Debiec J, Monfils MH, Schafe GE, LeDoux JE.** Synapse-specific reconsolidation of distinct fear memories in the lateral amygdala. *Nat Neurosci.* 2007;10:414-416.
10. **Sim SE, Park SW, Choi SL, Yu NK, Ko HG, Jang DJ, Lee K, Kaang BK.** Assessment of the effects of virus-mediated limited Oct4 overexpression on the structure of the hippocampus and behavior in mice. *BMB Rep.* 2011;44:793-798.
11. **Flood JF, Rosenzweig MR, Bennett EL, Orme AE.** The influence of duration of protein synthesis inhibition on memory. *Physiol Behav.* 1973;10:555-562.
12. **Choi JH, Kim JE, Kaang BK.** Protein synthesis and degradation are required for the incorporation of modified information into the pre-existing object-location memory. *Mol Brain.* 2010; 3:1.
13. **Ben Mamou C, Gamache K, Nader K.** NMDA receptors are critical for unleashing consolidated auditory fear memories. *Nat Neurosci.* 2006;9:1237-1239.
14. **Lee SH, Choi JH, Lee N, Lee HR, Kim JI, Yu NK, Choi SL, Lee SH, Kim H, Kaang BK.** Synaptic protein degradation underlies destabilization of retrieved fear memory. *Science.* 2008;319:1253-1256.
15. **Mamiya N, Fukushima H, Suzuki A, Matsuyama Z, Homma S, Frankland PW, Kida S.** Brain region-specific gene expression activation required for reconsolidation and extinction of contextual fear memory. *J Neurosci.* 2009;29:402-413.